STIMULI-RESPONSIVE POLYMER-GRAFTED LIPID-BASED COMPLEX FLUIDS AS ORGANIZED MEDIA FOR SUPRAMOLECULAR PHOTOCHEMISTRY

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INTRODUCTION

Recent studies involving the use of micelles, reverse micelles, and microemulsions as organized microheterogeneous media for effecting photochemical transformations have led to growing recognition that the nature of the reaction medium (*i.e.*, microenvironment) may strongly influence the course and efficiency of photoinduced electron transfer (1,2). Of particular interest in photochemical energy conversion research is the study of such effects in natural photosynthesis, the process whereby plants and photosynthetic bacteria convert light into chemical energy. The primary process in photosynthesis occurs in transmembrane pigment-protein complexes called reaction centers (RCs), where following the absorption of light, primary charge separation occurs. This electrochemical energy is stored, and the initial charge separation used to drive all subsequent electron and proton transfer reactions in photosynthesis.

The bacterial photosynthetic reaction center provides an excellent protein-pigment complex with which to explore the influence of environment on biological electron transfer, as it is a membrane-associated protein that has been structurally well-characterized and that can be readily isolated from natural photosynthetic membranes and purified (3). One area of current research interest is the determination of the effect of conformational changes in detergent-solubilized RCs on electron transfer (4). In this report, we consider a related issue: the introduction of RCs into biomembrane mimetics and its impact on protein conformation, orientation, and function. As a medium for these studies, we have employed a recently developed a stimulus-responsive complex fluid (smart material) that possesses the ability to respond to an environmental change/external stimulus on a molecular level and amplify it in the form of a macroscopically observable phase/structural change (5). Specifically, this material undergoes a dramatic, thermoreversible phase change from a fluid, micellar phase to a biomembrane-mimetic, lamellar gel phase as the temperature is raised above 16°C. Macroscopically, this phase change manifests itself as a change from a low-viscosity state with no detectable optical birefringence to a high-viscosity (gel) state that shows strong birefringence (i.e., liquid crystal). This complex fluid comprises three organic solids: a zwitterionic surfactant, a phospholipid, and a PEGylated phospholipid that spontaneously self-assembles into a non-covalent aggregate when dispersed in water. The inverted phase behavior (*i.e.*, a fluid phase at a lower temperature than the liquid-crystalline gel phase) of this composition is unique, and allows delicate biological macromolecules (*e.g.*, membrane proteins) to be introduced/dispersed in the complex fluid at low temperature and transferred to the ordered, liquid-crystalline phase by simply warming to room temperature. This material thus offers new opportunities for conducting studies of supramolecular photochemistry, for performing fundamental investigations on proteins such as RCs in native-like environments, and for examining how protein functioning is modulated by the surrounding medium. In addition, this complex fluid offers the potential for harnessing the native functioning of biological molecules for the development of protein-based devices for electro-optic and/or solar energy conversion by spatially organizing them in a synthetic matrix.

EXPERIMENTAL

Preparation of Reaction Centers and Complex Fluids. Membrane-associated, photosynthetic reaction centers (RCs) from the photosynthetic bacterium *Rhodobacter sphaeroides* R-26 were isolated and purifed using established procedures (6). Briefly, the RCs were extracted from chromatophores with a solution consisting of 0.6% (w/v) LDAO, 100 mM NaCl, and 10 mM Tris at pH 7.8, and partially purified by ammonium sulfate fractionation followed by sucrose density gradient centrifugation. Final purification was achieved on a DEAE Sephracel column by extensive washing with a solution of 0.06% (w/v) LDAO, 60 mM NaCl, and 10mM Tris at pH 7.8. Purified RCs were eluted from the column with 0.06% (w/v) LDAO, 280 mM NaCl, 10 mM Tris pH 7.8. RCs were dialyzed against 100 mM NaCl, 10 mM Tris pH 7.8, 0.1% LDAO prior to introduction into the complex fluid. The complex fluid was prepared by hydration of the organic components, dimyristoylphosphatidylcholine (DMPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-N-poly(ethylene glycol) (DMPE-EO_{45}), and lauryldimethylamine-N-oxide (LDAO) using the composition reported previously (5).

Physical Methods. UV-visible-NIR absorption spectra were recorded using a Shimadzu 1601 spectrophotometer at a spectral resolution of 2 nm. Time-resolved spectroscopic measurements were made with a single-beam, pump-probe, diode array instrument following procedures previously described (7). Synchrotron small angle X-ray scattering (SAXS) measurements were preformed either on the BESSRC undulator beamline (12ID) of the Advanced Photon Source (APS) at Argonne National Laboratory or at the Stanford Synchrotron Radiation Laboratory (SSRL, Stanford, CA) on beamline 4-2. The scattering profiles were recorded at APS were made with a mosaic detector composed of 9 CCD chips with an imaging area of 15 x 15 cm, with 1536 x 1536 pixel resolution. The scattering curves collected at SSRL recorded using a linear detector (BioLogic, Grenoble, France). The sample-to-detector distances were set such that the detecting range for momentum transfer was $0.006 < Q < 0.3$ Å^{-1} at SSRL and $0.008 < Q < 0.8$ Å^{-1} at APS. Samples were held in 1.5 mm quartz capillaries or in 1mm pathlength plexiglass cells with quartz coverslips as windows. Data were corrected for background scattering and calibrated based upon the known positions of silver behenate powder Bragg reflections. Small angle neutron scattering was performed on the SAND beamline at the Intense Pulsed Neutron Source (IPNS), at Argonne National Laboratory. The average wavelength of radiation was 1-14 Å and the momentum transfer, Q was 0.0035 - 0.6 Å. Samples were held in sealed, 1mm pathlength quartz cells. Sample temperature was regulated by a water recirculating bath and measured using a calibrated thermocouple. Low temperature measurements were carried out with the sample chamber under a N_2 atmosphere. Samples were prepared with D_2O, thereby eliminating the large incoherent background arising from H_2O and enhancing the contrast between the lipid aggregates and the solvent. Data were collected with the sample-to-detector distance of 1.54 m. Scattered neutrons were measured using a 128 x 128 array of position-sensitive, gas-filled 40 x 40 cm proportional counters, while the wavelengths were measured by time-of-flight. Data were collected for 5-8 h and reduced using standard methods (9).

RESULTS AND DISCUSSION

In our initial investigations, the effect of introducing RCs into the stimuli-responsive complex fluid on the extent of denaturation of the protein and its functional properties were examined. Introduction of the protein (as a solution in its detergent-solubilized state) into the preformed complex fluid produced an optically transparent, deep blue gel at room temperature. Evaluation of the extent of retention of the native structure and photochemical activity of the reconstituted RCs was carried out by two spectroscopic assays. First, the stability of reaction centers within the gel phase of the complex fluid was determined by monitoring the position and relative intensities of the co-factor absorption bands. The photosynthetic reaction center from *Rhodobacter sphaeroides* R26 is a large protein (10^5 kD) composed of three protein subunits (L, M, H) encasing nine co-factors arranged in *ca.* C_2 symmetry (3) (Figure 1B, C). Two bacteriochlorophyll molecules comprise the special pair that functions as the primary electron donor. On each side of this dimer is a bacteriochlorophyll monomer (Bchl) that, in turn, lies adjacent to a bacteriopheophytin (Bph), followed by a ubiquinone. A non-heme iron separates the primary quinone (Qa) from the terminal acceptor, the secondary quinone (Qb). The room temperature, ground-state absorption spectrum of the reaction center in the near-IR region shows three major absorbance bands, the position and intensities of which are sensitive to the integrity of the co-factors and local protein environment.

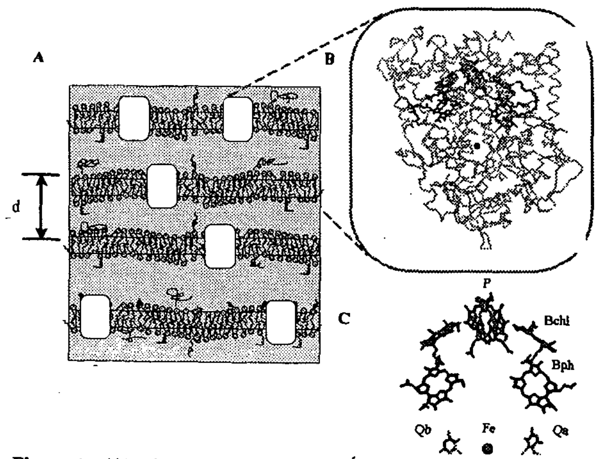

Figure 1. (A). Schematic showing lamellar structure of polymer-grafted lipid-based complex fluid with possible arrangement of reconstituted RCs. (B). The molecular structure of the photosynthetic reaction center (RC) of purple bacteria (*Rhodobacter sphaeroides* R26). (C). Co-factor arrangement of RCs taken from X-ray crystal structure data (3).

These absorption peaks, whose intensities are *ca.* 1:2:1, arise from the bacteriopheophytin (Bph, 755 nm), monomeric bacteriochlorophyll (Bchl, 802 nm), and bacteriochlorophyll dimer (P, 865 nm) cofactors. A comparison of the NIR absorption spectra of reaction centers in their detergent solubilized state and after their reconstitution into the lamellar gel phase of the complex fluid is presented in Figure 2. The close correspondence between the two spectra demonstrates that reaction centers can be successfully incorporated into the complex fluid without loss of structural integrity.

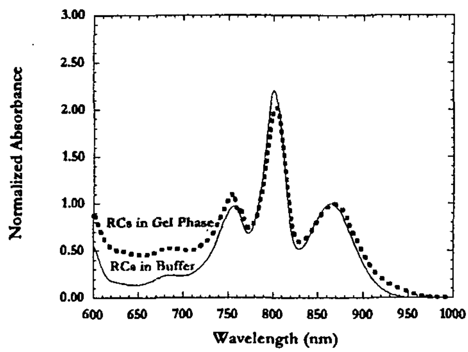

Figure 2. NIR absorption spectra of RCs in 10mM Tris-100mM NaCl-0.01%LDAO-0.01mM EDTA-pH7.8 buffer solution (solid line) and in polymer-grafted membrane-mimetic gel (dashed line).

The second spectroscopic assay involved evaluation of the photosynthetic activity of the RC in the gel phase of the complex fluid. A well-documented, diagnostic assay for reaction center photochemistry is the measurement of the laser flash-induced, transient absorbance decrease and recovery of the 865 nm absorption band of the primary electron donor chlorophyll, P (7). That is, following laser-induced, ps charge separation, the photosynthetic reaction ends with electron transfer between quinone cofactors (accompanied by proton uptake), Q_A and Q_B:

$$PQ_AQ_B + h\nu \rightleftharpoons P^+Q_A^-Q_B \rightleftharpoons P^+Q_AQ_B^-.$$

This electron transfer (between quinones) is the final such transfer in the reaction center, and the one that is the most sensitive to perturbation, since Q_B is a readily exchangeable cofactor. The recovery of the ground state P absorption following laser flash excitation has characteristic lifetimes that depend upon the extent of completion of the photochemical sequence. Kinetics for laser flash-induced recovery of the 865 nm band measured both for RCs in the native, detergent-solubilized state and in the gel phase of the complex fluid are shown in Figure 3. Recovery of the 865 nm band in the native RCs was fit as a biexponential function, with 90% of the recovery having a lifetime of 0.9 s, corresponding to recombination from the final $P^+Q_B^-$ state, and the remainder having a lifetime of 60 ms, corresponding to recovery from the $P^+Q_A^-$. In the gel sample, 80% of the recovery was fit with a lifetime of 1.4 s, and the remaining 20% was fit with a 120 ms lifetime. The recovery kinetics indicate that extent of the final $Q_A^-Q_B \rightarrow Q_AQ_B^-$ electron transfer was diminished only by about 10% in the gel phase. The increase in the recovery times for the $P^+Q_B^-$ and $P^+Q_A^-$ in the gel phase is consistent with previous measurements that showed similar recovery times for RCs in high osmolarity media (7). These results indicate that the polymer-grafted, membrane-mimetic matrix has no adverse impact upon the electron and proton transfer activities of the RC.

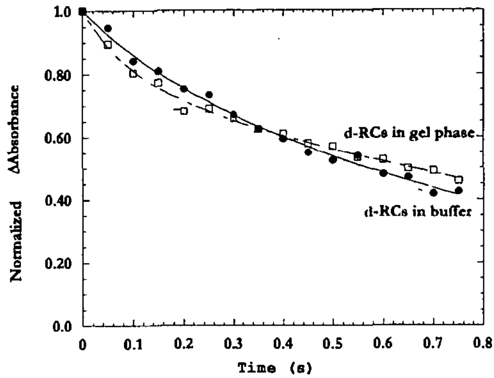

Figure 3. Kinetics for laser-flash induced recovery of the 865 nm band for RCs in 10mM Tris-100mM-NaCl-0.01%LDAO-0.01mM EDTA-pH7.8 buffer solution (solid circles) and in polymer-grafted membrane-mimetic gel (open squares).

To determine the effect of the introduction of RCs on the properties of the complex fluid, the response of the gel phase to addition of the RCs was examined by small angle X-ray diffraction. A typical SAXS pattern recorded for the complex fluid in the gel phase (at room temperature) is presented in Figure 4A. The pattern is dominated by four Bragg peaks of integral order (Q = 0.041, 0.083, 0.124, 0.164 $Å^{-1}$) and is indicative of a lamellar structure, that is, one consisting of alternating layers of water and organic components (Figure 1A). The first order Bragg peak corresponds to a periodicity of 153 Å. The effect of the incorporation of RCs (12 μm) into the gel phase is presented in Figure 4B. The SAXS profile shows the same periodic structure (*i.e.*, lamellar structure) as indicated by the integral order of the four observed Bragg peaks (Q = 0.035, 0.072, 0.108, 0.144 $Å^{-1}$). Some changes in the scattering pattern are apparent, however. First, the position of the first order Bragg peak indicates that the lattice spacing increases from 153 to 180 Å upon protein reconstitution. Second, introduction of RCs, which is believed to occur by insertion into the alkane region of the lipid bilayer, leads to both a reduction in the amplitude and an increase in the breadth of the diffraction peaks, suggesting a loss of spatial coherence and orientational disorder. This finding is consistent with prior work in which hydrophobic, dodecanethiol- derivatized gold nanoparticles, which selectively partition into the alkyl chains of the lipid/surfactant bilayer, were found to produce a similar effect on the observed SAXS profile (10).

Small angle neutron scattering (SANS) has also been used to assess the gels response to RC reconstitution and to obtain information regarding structural organization. Unlike X-ray scattering techniques, SANS permits determination of the structure of the fluid (cold) phase, by selective deuteration to highlight the aqueous channels (8). Previous, neutron scattering results yielded a low-resolution structure of the cold phase as normal hexagonal (5). Preliminary SANS investigations on complex fluid compositions incorporating reaction centers (data not shown) indicate that the cold phase structure changes to a cubic phase upon RC addition. Details of this analysis, along with results of studies employing deuterated reaction centers used to assess reaction center organization within these phases, will be presented in a future report (11).

CONCLUSIONS

The results presented here indicate that introduction of bacterial photosynthetic reaction centers into the polymer-grafted lipid-based complex fluid has no adverse impact on the structural or functional integrity of the protein. Preliminary investigations indicate that RC reconstitution does alter the structural organization of the complex fluid below the phase transition temperature (from a 2-D hexagonal array of prolate micelles to a 3-D cubic structure of micelles). Conversely, this introduction does not appreciably alter the structural properties of the gel phase of the complex fluid. Taken together, these results demonstrate that the complex fluid provides a medium of great potential utility in which to conduct fundamental investigations of protein structure and function.

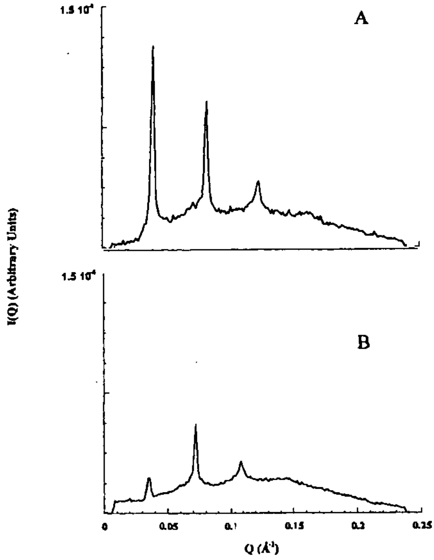

Figure 4. Small angle X-ray scattering profiles of (A) gel phase of the complex fluid, (B) gel phase incorporating 12 μm RCs.

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